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The Role of Cancer Stem Cells in the Initiation and Propagation of Tumorigenesis

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Cloning of the Genes for T84.66, an Antibody That Has a High Specificity and Affinity for Carcinoembryonic Antigen, and Expression of Chimeric Human/Mouse T84.66 Genes in Myeloma and Chinese Hamster Ovary Cells¹

Michael Neumaier, Louise Shively, Fun-Shan Chen,
Franz-Josef Gaida, Christine Ilgen, Raymond J. Paxton,
John E. Shively² and Arthur D. Riggs

Divisions of Biology [L. S., C. I., A. D. R.] and Immunology [F.-S. C., R. J. P., J. E. S.], Beckman Research Institute of the City of Hope, Duarte, California 91010, and Department for Clinical Chemistry and Pathobiochemistry, University of Technology, D-5100 Aachen, Federal Republic of Germany [M. N., F.-J. G.]

Carcinoembryonic antigen (CEA) is one of the best characterized tumor-associated antigens and is extensively used in the *in vitro* immunodiagnosis of human colon adenocarcinomas. Among a number of anti-CEA monoclonal antibodies, the murine monoclonal antibody T84.66 shows the highest specificity and affinity for CEA and has been used successfully for *in vivo* tumor imaging in mice and humans. We report here the cloning and sequencing of the genes coding for monoclonal antibody T84.66 and the amino acid sequence of the variable regions for the heavy and light chains. We also report the construction of mouse/human chimeric IgG1 antibody genes using T84.66 variable region genes and human constant region genes. The resulting chimeric gene constructs were transfected into murine myeloma cells (Sp2/0) by electroporation and into Chinese hamster ovary cells by lipofection. The chimeric antibodies obtained exhibited the same specificity and affinity for CEA as that of the T84.66 immunoglobulin produced by the murine hybridoma cell line. Antibody concentrations in culture medium supernatants were clonally variable but similar (15–480 ng/ml) for both Sp2/0 and Chinese hamster ovary transfectants; the average production by Chinese hamster ovary transfectants was only 3–5-fold less than Sp2/0 transfectants. Ascites production of Sp2/0 transfectants is sufficiently high (900 µg/ml) for initial *in vivo* studies with humanized T84.66.

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² To whom requests for reprints should be addressed, at Division of Immunology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010-0269.

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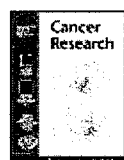
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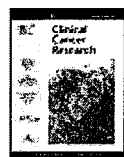
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